

# Suppression of Antigen-Specific Lymphocyte Activation in Simulated Microgravity

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**Running Title: Antigen-Specific Activation in Simulated Microgravity**

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**Abbreviations:** BAT, Bb-specific T cell line; Bb, *Borrelia burgdorferi*; HARV, high-aspect rotating vessel; HBSS, Hanks' balanced salt solution; MLR, mixed-lymphocyte reaction; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; RWV, rotating wall vessel; TT, tetanus toxoid.

## ABSTRACT

Various parameters of immune suppression are observed in astronauts during and after spaceflight, and in isolated immune cells in true and simulated microgravity. Specifically, polyclonal activation of T cells is severely suppressed in true and simulated microgravity. These recent findings with various polyclonal activators suggests a suppression of oligoclonal lymphocyte activation in microgravity. We utilized rotating wall vessel (RWV) bioreactors that simulate aspects of microgravity for cell cultures to analyze three models of antigen-specific activation. A mixed-lymphocyte reaction (MLR), as a model for a primary immune response; a tetanus toxoid (TT) response and a *B. burgdorferi* (Bb) response, as models of a secondary immune response, were all suppressed in the RWV bioreactor. Our findings confirm that the suppression of activation observed with polyclonal models also encompasses oligoclonal antigen-specific activation.

**Keywords:** spaceflight, space immunology, gravitational biology, immune suppression

## INTRODUCTION

Various parameters of immunity are suppressed both during and shortly after spaceflight [1]. Notably, the ability of astronauts in orbit to manifest a delayed-type hypersensitivity (DTH) response to common recall antigens is compromised [2, 3]. Psychological and physical stress, cosmic radiation, and microgravity may play a role in the suppression of immune performance during spaceflight [4]. Although stress is a significant factor, studies using isolated lymphoid cells suggest a role for microgravity alone [5, 6]. However, the in vitro functioning of primary and secondary immune responses in microgravity is unknown.

There are several methods for modeling microgravity on Earth for cell cultures. We utilized the rotating wall vessel (RWV) bioreactor to simulate aspects of microgravity. The RWV bioreactor adapts clinostatic technology and rotates a solid fluid body around a horizontal axis at a constant speed. This compensates gravity by rotation and places cells within the fluid body into a sustained free-fall [7, 8]. The RWV bioreactor is a reliable predictor of microgravity-related phenomena since suppression of lymphocyte locomotion through collagen matrices observed in the RWV bioreactor was also observed in the true microgravity of orbit [9]. The RWV bioreactor was also used to analyze the suppressed response of T cells to mitogens [6]. Therein, signal transduction upstream of protein kinase C was sensitive to simulated microgravity.

We employed three antigen-specific activation models to assess antigen-specific performance: the mixed-lymphocyte reaction (MLR), tetanus toxoid (TT) responsiveness, and the response of a Bb-specific T cell line (BAT) to Bb.

## MATERIALS AND METHODS

*Cells.* Peripheral blood mononuclear cells (PBMC) were isolated from human buffy coats (Gulf Coast Regional Blood Center, Houston, TX) on Ficoll-Hypaque gradients (Pharmacia LKB, Piscataway, PA), washed 3X in Hanks' balanced salt solution (HBSS), and resuspended in RPMI-AB (RPMI-1640, GibcoBRL, Grand Island, NY; 10% heat-inactivated male human AB<sup>+</sup> serum, Sigma, St. Louis, MO; and penicillin, 100 U/ml, -streptomycin, 100 µg/ml, GibcoBRL). The murine anti-Bb specific T cell line (BAT) is previously described [10]. Briefly, the BAT cell line was developed by immunizing female C3H/HeNcr (MTV<sup>-</sup>) mice with inactivated low-passage *B. burgdorferi* sensu stricto isolate B31. The BAT cells are CD4<sup>+</sup>, Th1 phenotype, and were maintained in RPMI-1640 with 10% heat-inactivated FBS (Hyclone Labs, Logan, UT).

*Bioreactors.* The RWV bioreactor (Synthecon, Houston, TX) is a cylindrical culture vessel with zero head-space and a silicon membrane for direct gas exchange that is horizontally rotated (14 rpm). Both 55-ml RWVs and 10-ml HARVs (high-aspect rotating vessel) were used for this study. The HARV is similar to the RWV but with slightly different vessel dimensions. The operation of these vessels is described previously [6, 7, 9]. Due to the limited number of bioreactors and cells, most experiments were not performed simultaneously with triplicate RWVs. Instead, experiments were repeated independently two to three times. The data shown are from one representative experiment.

*One-way MLR.* PBMC ( $1 \times 10^6$  cells/ml) from two mismatched donors were mixed in RPMI-AB in both 55-ml RWVs and T-75 T-flasks for six days. The stimulator cells were treated with 25 µg/ml mitomycin C for 30 min. Mixed PBMC were also incorporated into collagen beads.

*Tetanus toxoid model.* PBMC ( $1 \times 10^6$  cells/ml) were stimulated with 10  $\mu$ g/ml TT (column purified, Mass Biologic Labs, Jamaica Plain, MA) in RPMI-AB in both 10-ml HARVs and 10-ml T-flasks for six days. To minimize background proliferation, prior to TT stimulation some monocytes were removed from the PBMC population by adherence to plastic. PBMC were also incorporated into collagen beads and stimulated in a 55-ml RWV and a T-75 T-flask.

*B. burgdorferi model.* BAT cells obtained after their rest cycle, freshly isolated and irradiated syngeneic murine splenocytes (to act as APCs), and inactivated Bb (to act as antigen) were prepared as previously described [10]. Reactions were initiated in both suspension cultures and in collagen bead cultures for 48 h. In suspension,  $5 \times 10^4$  cells/ml BAT cells,  $5 \times 10^4$  cells/ml APCs, and 5  $\mu$ g/ml Bb were cultured in complete RPMI in 10 ml HARVs and 10 ml T-flasks. Collagen bead cultures were set up with  $1 \times 10^5$  BAT cells/bead,  $1 \times 10^6$  APCs/bead, and 0.125  $\mu$ g/bead Bb in 55 ml RWVs and T-75 T-flasks with approximately 60 beads per vessel.

*Collagen beads.* To promote cell-cell/substratum interaction PBMC and BAT cells were incorporated into 25- $\mu$ l collagen beads. Collagen solutions [11, 12] and beads [6] were prepared. Vessels were seeded at a concentration of approximately 60 beads per vessel. Isolated beads were washed briefly with HBSS on Falcon nylon cell strainers (Fisher, Houston, TX) and then digested with a collagenase cocktail [6]. The cells were collected by centrifugation and washed 2 X in RPMI.

*Proliferation assay.* Proliferation was determined for sampled cells by [ $^3$ H]thymidine incorporation. Sampled cells ( $2 \times 10^5$  cells/well) were labeled with [methyl- $^3$ H]thymidine (1 $\mu$ Ci/well, 5 Ci/mmol, Amersham Life Sciences, Arlington Heights, IL) in triplicate for 18 h in standard 96-well plates, harvested onto glass filter paper, and analyzed by standard liquid

scintillation techniques. The data are presented as ? cpm (experimental cpm minus background cpm).

## RESULTS AND DISCUSSION

*The MLR is suppressed in the RWV bioreactor.* A one-way MLR was established by culturing PBMC from one donor with mitomycin C treated PBMC from a mismatched donor for 6 days. Cells were cultured in suspension or in small polymerized collagen beads. Cells in collagen beads have increased cell-cell/substratum interactions which reverses some of the suppressive effects of simulated microgravity [6]. The proliferation of lymphocytes in the MLR was suppressed in the RWV bioreactor (Fig. 1). Culture of cells in collagen beads slightly restored proliferation.

*The response of PBMC to TT is suppressed in the RWV bioreactor.* Most people in North America have been immunized with TT, and therefore, there should be some TT reactive T cells in the PBMC of donors. PBMC cultured in the RWV bioreactor failed to respond to TT (Fig. 2). Unlike similar experiments with PHA [6] and the MLR, the polymerization of PBMC within collagen beads did not afford any proliferation.

*The response of T cells to Bb is suppressed in the RWV bioreactor.* *B. burgdorferi* is a spirochete and the causative agent of lyme disease [13]. Following a three week rest period after their last stimulation, BAT cells were cultured with killed Bb and irradiated syngeneic murine splenocytes to act as APCs for 48 hours. The BAT cells failed to proliferate in response to the

Bb in the RWV bioreactor (Fig. 3). As in the TT study, the polymerization of BAT cells within collagen beads did not afford any proliferation.

Numerous studies have examined polyclonal activation in both real and simulated microgravity. Yet, *in vitro* antigen-specific activation of T lymphocytes has not been assessed in any form of microgravity at a cellular level. However, both the Russian and U.S. space programs demonstrated suppressed DTH responses to recall antigens in astronauts on orbit [2, 3]. In addition to the stress hormone related immune suppression, the inability of T cells to activate in response to the recall antigens may play a role in the suppressed response. We found primary and secondary antigen-specific activation of T cells to be suppressed in simulated microgravity and thus, confirmed the significance of the suppression of polyclonal activation. Unlike PHA activation, there was little restoration of proliferation in secondary antigen-specific activation performed with cells within collagen beads. It is not clear why adherence of the cells to collagen in these experiments did not help them resist the effects of simulated microgravity.

The results from the PHA studies suggest that microgravity interferes with early signal transduction events in the cell membrane. These are common mechanisms to both primary and secondary immune responses. We found no difference in the ability of simulated microgravity to suppress T cell activation if the antigen was soluble or an intact cell. Whether the antigen was a soluble protein (TT), an intact microorganism (Bb), or an intact mammalian cell (MLR), there was no effect on the suppression of activation. Again this is consistent since the microgravity-induced lesion seems to be associated with a basic signal transduction mechanism utilized by T cells regardless of the state of the antigen.

We extended the analysis of T cell activation in microgravity to antigen-specific forms of activation and described its suppression. Our data suggest that microgravity may suppress activation throughout the T cell subpopulation.

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## Figure Legends

**FIG. 1.** MLR in the RWV bioreactor. Mismatched PBMC were co-cultured for six days in 55-ml RWVs and T-75 T-flasks. The cells were either cultured in suspension or encapsulated within 25- $\mu$ l collagen beads. After six days, proliferation of PBMC sampled from beads and vessels was assayed. The data are representative of three experiments.

**FIG. 2.** Response of PBMC to TT in the RWV bioreactor. PBMC were stimulated with TT in a 55-ml RWV and a T-75 T-flask (encapsulated within small collagen beads) or in a 10-ml HARV and a 10-ml T-flask (in suspension). After six days, proliferation of PBMC sampled from beads and vessels was assayed. The data are representative of three experiments.

**Figure 3.** Response of anti-*Bb* T cell line (BAT) to Bb in the RWV bioreactor. BAT cells were stimulated with killed Bb in a 55-ml RWV and a T-75 T-flask (encapsulated within small collagen beads) or in a 10-ml HARV and a 10-ml T-flask (in suspension). After 48 h, proliferation of BAT cells sampled from beads and vessels was assayed. The data are representative of two experiments.

Fig 1.

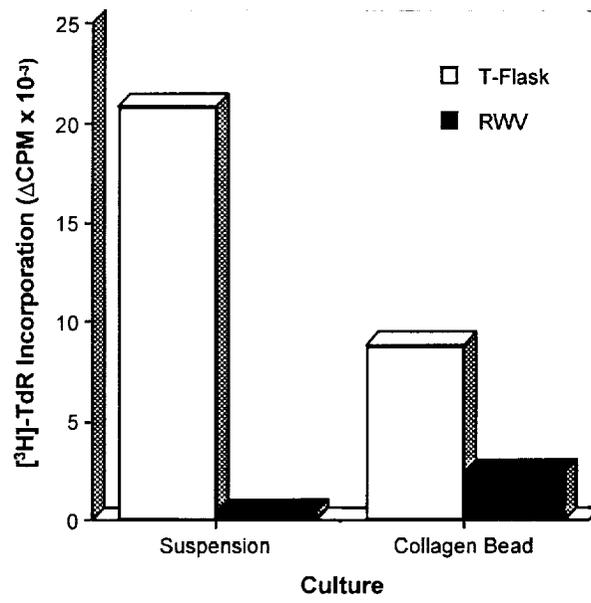


Fig. 2

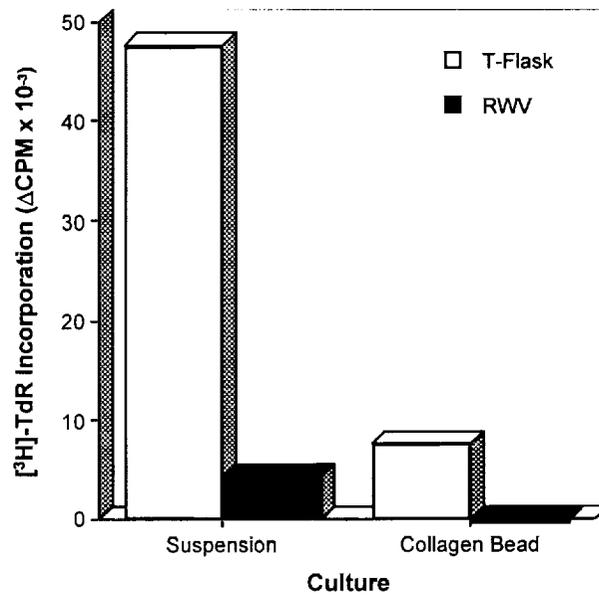


Fig. 3

